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(54) Title: BIS PHENOL OR PHENOZY COMPOUNDS FOR IMMUNE MODULATION

(57) **Abstract**

Disclosed are chemical agents for modulating certain cellular immune reactions that can lead to autoimmune disorders. By specific modulation, harmful immune reactions can be lessened in severity or even prevented without resorting to potentially dangerous general immune suppression. The described chemical agents inhibit IL-12 induction of the secretion of key immune modulators. The described chemical agents are specific inhibitors of IL-12 induced Th1 immune response.

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SPECIFICATION

BIS PHENOL OR PHENOZY COMPOUNDS FOR IMMUNE MODULATION

Technical Field and Background Art

Autoimmune diseases result from the recognition of "self" by the immune system followed by a humoral (antibody) or cell - mediated response that leads to the destruction of the one's own cells. While a healthy immune system selects against self - reactive immune cells (T cells and B cells) in the thymus by quickly destroying them, this system is imperfect. When self - reactive cells are released into the circulation and penetrate into peripheral tissues they may encounter the self antigen to which they can respond. These antigens are displayed on the surface of cells in the form of peptide fragments, non - covalently associated with either class I or class II antigen molecules of the major histocompatibility complex (HLA in humans). Fortunately, this first encounter of self reactivity generally results in a weak response. This is because multiple signals are required to stimulate a proliferative response that both activates the effector function of the cell and increases its number by cell division. Without a second co - stimulatory signal, which is found on professional antigen - presenting cells (APC), the T cell becomes non - reactive (or anergic) to a second exposure to the same antigen and a harmful reaction is prevented.

How this protective system breaks down in autoimmune disease is under intense investigation and many associations have been made between specific types of immune responses and disease activity. For example, conditions that lead to the up - regulation of HLA molecules in the central nervous system (CNS), and increased antigen presentation, have been associated with the T - cell mediated destruction of nervous tissue in multiple sclerosis (MS) patients. While the exact triggering event is unknown, a clear picture is emerging as to how the immune system regulates such responses. One of the key immune regulators is the T helper cell which reacts to antigens presented on HLA class II

molecules. This $CD4^+$ cell differentiates in response to antigenic stimulation and becomes a type 1 or type 2 helper (Th1 or Th2) according to the type of cytokines that it secretes (Mosmann and Coffman, Ann. Rev Immunol. 7:145). A Th1 response leads to the secretion of interleukin - 2 (IL - 2) and interferon - γ (IFN - γ) which stimulates cell - mediated immune reactions against intracellular pathogens. A Th2 response leads to the secretion of IL - 4, IL - 5 and IL - 10 which stimulates antibody responses to extracellular pathogens. The most interesting component of this system of regulation is that one response inhibits the other through the negative regulatory activities of the cytokines that are produced. Thus, IL - 4 and IL - 10 can down - regulate Th1 responses while IFN - γ can down - regulate Th2 responses.

The importance of such a regulatory feedback loop in autoimmune disease recently has been associated with disease activity in multiple sclerosis. T cells cloned from patients undergoing active disease have been shown to produce Th1 cytokines upon stimulation with antigen *in vitro* (Correale et al. J. Immunol. 154:2959). T cell clones obtained from the same patient during the remission phase produce Th2 cytokines. They also produce another potent suppressor of cell- mediated immunity, tumor growth factor β (TGF - β).

The regulatory activity of T helper cells and their differentiation following exposure to antigen is regulated by cytokines as well. IL - 12 has been shown to be essential in the generation of Th1 cells. IL - 12 is released primarily by the antigen presenting cell, which for Th1 responses is normally a macrophage (reviewed by Trinchieri, Blood 84:4008). Other cytokines also are secreted by the responding T cell after antigen stimulation, especially IL - 2. Cytokines IL - 12 and IL - 2 have a powerful synergistic effect in the induction of IFN - γ from both T helpers and natural killer (NK) cells (Eur. Patent Appl. 90123670.3). This secreted IFN - γ then inhibits any Th2 cell proliferation and polarizes the response to favor cell - mediated immunity (the Th1 response). If IL - 4 was the major cytokine present during antigen stimulation, a Th2 response would be made and the Th1 response would be inhibited. Thus, the initiating event that establishes the cytokine environment has an important role in determining the

nature of the immune response. Another consequence of the Th phenotype is reflected in the isotype of antibody that is made in response to an antigen. Th1 responses lead to increases in cytolytic antibodies, i.e., those capable of mediating antibody - dependent cellular cytotoxicity (ADCC) and those that activate the complement system. Th2 responses lead to the production in non - cytolytic classes (isotypes) of antibodies. The importance of this phenomenon has recently been described in mouse models of collagen - induced arthritis where Th1 responses induced by IL - 12 favored the production of IgG2a (cytolytic) over IgG1 (non - cytolytic) and this class switching correlated with disease occurrence and severity (Germann et al., Proc. Natl. Acad. Sci. 92:4823).

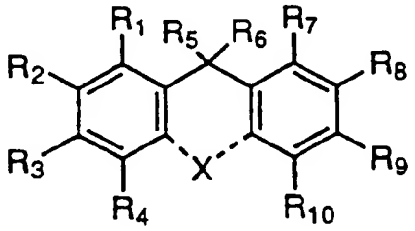
It is one object of this invention to provide compositions for antagonizing the IL - 12 induced activities of immune cells. It is another object of this invention to provide a method for antagonizing IL - 12 induced activities of T helper cells so as to inhibit the IFN - γ induced Th1 response, be effective to modulate the induction of Th2 cells; be effective to inhibit a cellular immune response; and/or be effective to stimulate the production of Th2 cytokines including IL4, IL - 5, and IL - 10. It is still another object of the invention to provide a method for stimulating the cellular production of cytokines in immune cells, which can be immune cells in a mixed or selected population of cells in an *in vitro* cell culture or can be circulating immune cells in a mammal. It is still another object of the invention to provide an *in vitro* diagnostic for measuring IL4 production in peripheral blood mononuclear cells.

Disclosure of the Invention

Certain bis phenol or phenoxy compounds, and derivatives thereof, have been discovered to antagonize the IL - 12 mediated induction of IFN - γ synthesis. The bis - compounds also have been discovered to be therapeutically useful in the stimulation of the immune system to inhibit the IL - 12 induced production of IFN γ and thereby to modulate induction of Th2 cells. Inhibition of IFN γ production can inhibit the induction of Th1 immune cells, and/or inhibit a cellular immune response. Modulation of the induction of Th2 cells can lead to stimulation of the secretion of Th2 cytokines including IL - 4, IL - 5,

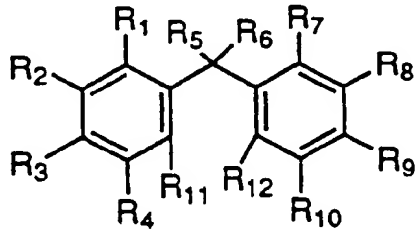
and IL - 10, to stimulation of the Th2 immune response against extracellular pathogens, and to the induction of antibody synthesis.

In one aspect, the invention is embodied as a composition for antagonizing IL-12 induced immune response. The composition comprises a pharmaceutically acceptable carrier and a bis- compound having the following general formula.



The X is optional, and if present, is -O-, -S-, or $-CH_2-$. At least one of R_1, R_2, R_3, and R_4, and at least one of R_7, R_8, R_9, and R_{10}, is OR_{13}, where R_{13} is H or lower alkyl. R_5 and R_6 are selected independently from H, C_1 - C_{12} branched or linear hydrocarbons, phenyl, phenyl substituted with halo, nitro, carboxy, alkoxy, hydroxy, alkoxy carbonyl, or alkyl, alkyl substituted with halo, nitro, carboxy, alkoxy, hydroxy, or alkoxy carbonyl, or alkenyl, alkenyl substituted with halo, nitro, carboxy, alkoxy, hydroxy, or alkoxy carbonyl. Each of R_1, R_2, R_3, R_4, R_7, R_8, R_9, and R_{10} which is not OR_{13} is independently hydrogen, halo or linear or branched lower alkyl. A lower alkyl means an alkyl group having 1 to 6 carbon atoms.

In another aspect, the invention is embodied as a composition for antagonizing the IL-12 induced immune response. The composition comprises a pharmaceutically acceptable carrier and a bis- compound having the following general formula.



At least one of R_1, R_2, R_3, R_4, and R_{11}, and at least one of R_7, R_8, R_9, R_{10} and R_{12}, is OR_{13}, where R_{13} is H or lower alkyl. R_5 and R_6 are selected independently from H, C_1 -

C_{12} branched or linear hydrocarbons, phenyl, phenyl substituted with halo, nitro, carboxy, alkoxy, hydroxy, alkoxy carbonyl, or alkyl, alkyl substituted with halo, nitro, carboxy, alkoxy, hydroxy, or alkoxy carbonyl, or alkenyl, alkenyl substituted with halo, nitro, carboxy, alkoxy, hydroxy, or alkoxy carbonyl. Each of R_1, R_2, R_3, R_4, R_7, R_8, R_9, R_{10}, R_{11} and R_{12} which is not OH or OR_{13} is independently hydrogen, halo or linear or branched lower alkyl.

Preferably, at least one of R_1, R_2, R_3, R_4, R_{11} and at least one of R_7, R_8, R_9, R_{10}, R_{12} is OH. One or both of R_5 and R_6 can be H or a hydrocarbon radical having 1 to 12 carbon atoms. More preferably, one of R_5 and R_6 is H and the other is a linear or branched alkyl chain having 1 to 12 carbon atoms. Each R_1, R_2, R_3, R_4, R_7, R_8, R_9, R_{10}, R_{11} and R_{12} which is not OH can be independently hydrogen, methyl, ethyl, propyl, isopropyl, butyl, tertiary-butyl, or linear or branched pentyl.

In another aspect the invention is embodied as a method of antagonizing the IL-12 mediated induction of IFN-γ synthesis in a mammal comprising the step of administering to a mammal one of the above described bis-compounds in an amount effective to antagonize IL-12 mediated induction of IFN-γ synthesis. Inhibition of IL-12 induced IFN-γ synthesis can have the effect of inhibiting the induction of Th1 cells; modulating the induction of Th2 cells; inhibiting a cellular immune response, and/or stimulating the production of Th2 cytokines including IL-4, IL-5, or IL-10.

In another aspect, the invention provides a method of antagonizing IL-12 in immune cells comprising contacting immune cells with a bis-compound, described above, in an amount sufficient to inhibit IL-12 induced production of IFN-γ. The immune cells which are contacted with the bis-compound can be a mixed population of cells in an *in vitro* cell culture, or can be a mixed population of cells which are circulating in a mammal.

Brief Description of the Drawing

Fig. 1 is a graph depicting the percent inhibition of γ-IFN production (% INH of γ-IFN), the percent inhibition of mixed lymphocyte reaction (% INH of MLR), and

the percent viability of the mixed lymphocytes (% Viability) after treatment with bis - phenol compound # 51853.

Fig. 2 is a graph depicting the percent inhibition of γ - IFN production (% INH of γ - IFN), percent inhibition of mixed lymphocyte reaction (% INH of MLR), and percent viability of the mixed lymphocytes (% Viability) after treatment with bis - phenol compound # 51852.

Fig. 3 is a graph depicting the percent inhibition of γ - IFN production (% INH of γ - IFN), percent inhibition of mixed lymphocyte reaction (% INH of MLR), and percent viability of the mixed lymphocytes (% Viability) after treatment with 8 - azaguanine (a control to show general immunosuppression).

Detailed Description of the Invention

The natural mechanism for combating inappropriate cell - mediated responses may be to suppress them with a Th2 - like response. The inhibition of IFN - γ , which is normally produced in the Th1 response, would then block many of its potentially harmful effects including the activation of macrophage, natural killer cells and cytolytic T cells, and the induction of class I and class II HLA in the target tissue.

The importance of such a "suppressor" effect in diseases such as MS is suggested by the finding that IFN - β , a molecule that down regulates the class II HLA induced by IFN - γ , reduces the number of relapses and the extent of central nervous system (CNS) inflammation (The IFNB Multiple Sclerosis Study Group, Neurology 43:655). The general immunosuppressing drug cyclosporine, on the other hand, can lessen autoimmune disease while it is administered, but increases its severity once the drug is withdrawn (Sorokin et al. J. Exp. Med. 164:1615). This is likely the result of inhibiting the suppressor effect as well as the undesirable cell - mediated response, such that the destructive response quickly returns, unchecked, as soon as the general immunosuppressing drug is no longer present.

One way of changing the outcome of an immune response would be to administer the appropriate cytokine at the time of antigen stimulation. The problem with

this approach is that the systemic administration of cytokines is difficult due to their very short circulating half - lives, their deleterious side effects, and their high cost of manufacture. Another approach is to identify small chemical inhibitors of either Th1 or Th2 cytokines so that the effective concentration of the non - inhibited cytokines is increased. For example, inhibitors of IL- 12 secretion or IL- 12 induced activities, e.g., the induction of IFN - γ , could selectively block cell - mediated immunity by preventing Th1 development. If this is done without inhibiting Th2 responses, then the produced Th2 cells could serve as suppressors of ongoing or future Th1 responses to the same antigen, as described above for MS (Corealle et al. (1995) J. Immunol., 154:2959 2968). This type of modulation of an immune response serves to stimulate the body's own protective mechanism against autoimmunity rather than suppressing the immune system altogether.

The present invention describes methods that can be used to identify small molecule inhibitors of Th1 immune responses that are not generally immunosuppressive, and discloses a claim of such selectively immunosuppressive compounds and compositions. The methods for synthesizing useful embodiments of the invention are described as well as assays useful for testing their pharmacological activities both *in vitro* and in pre - clinical *in vivo* animal models. The instant invention provides to derivatives of bis - compounds of the general formulae described herein.

Compounds which are particularly effective for each of these purposes include substituted bis - compounds and particularly bis - phenols which are described in detail herein. The terms "bis - compound(s)" and "bis - phenol compound" will be used herein to include all substituted bis - compounds herein described, and generally define a molecular structure comprising a variously substituted central carbon atom flanked by a pair of phenol or phenoxy groups, optionally derivatized as disclosed herein.

An effective amount of the bis - compound comprises an amount of the individual agent such that the desired clinical endpoint, antagonization of IL - 12 induced immune response, is reached. The amount to be administered will depend on the potency, bioavailability, *in vivo* half - life, and toxicity of the individual compound. In general, the dose would reasonably be expected to range between 0.1 to 100 mg/kg per adult per

administration, and preferably would be between 1 to 20 mg/ kg per adult per administration.

The language "therapeutically effective amount" is intended to include the amount or concentration of bis - compound sufficient to antagonize the IL - 12 induced immune response activities, such as inhibition of IFN γ . A therapeutically effective amount can be determined on an individual basis and will be based, at least in part, on consideration of the severity of the symptoms to be treated. Further, the effective amounts of the bis - compound may vary according to age, sex, and weight of the subject being treated. A therapeutically effective amount of a given bis - compound can be determined by one of ordinary skill in the art employing such factors described herein using no more than routine experimentation in clinical management.

In the preferred embodiments of each aspect of the present invention, the composition of bis - compound is prepared together with a pharmaceutically acceptable carrier substance for oral ingestion or parenteral injection. The language "pharmaceutically acceptable carrier" is intended to include substances capable of being co - administered with the bis - compound and which allows the compound to perform its intended function of antagonizing IL - 12. Examples of pharmaceutically acceptable carriers are commercially available inert gels or liquids. Gels comprise of the compound, a base selected from oleaginous base, water or emulsion - suspension base, and a gelling agent, such as hydroxypropyl cellulose, acrylic acid polymers, and the like. Liquids include emulsions, solutions, and suspensions. The term, "pharmaceutically acceptable salts" is intended to include salts which are recognized in the art. Typically these salts are capable of being hydrolyzed under physiological conditions. Examples of such salts include sodium, potassium, and hemisulfate. Additionally, a carrier having effective bioavailability should be used in preparations of the compound for oral ingestion.

The present invention further relates to pharmaceutical compositions containing the above noted bis - compounds and appropriate pharmaceutically acceptable excipients, oils such as corn oil, buffers such as PBS, saline, ethanol, polyethylene glycol, glycerin, polypropylene glycol, dimethylsulfoxide, and amide such a dimethylacetamide, proteins

such as albumin, a detergent such as Tween 80, mono- , oligo- , or polysaccharides, such as glucose, lactose, cyclodextrins and starch.

The composition may take forms such as suspensions, solutions, or emulsions in oily or aqueous vehicles, and may contain formulating agents, such as suspending, stabilizing, or dispersing agents, isotonic agents and/or dissolving co - solvents conventionally cited in the pharmaceutical art.

The term "subject" is intended to include all mammals such as humans, dogs, cats, horses, cows, goats, rats and mice.

The amounts of bis - compound incorporated into the formulation of the present invention is not critical; the concentration should only be in a range sufficient to permit ready application of the formulation in an amount which will deliver the desired amount of bis - compound.

Bis - phenol compounds are commercially available from chemical suppliers. Substituted bis - phenol compounds may be readily made by one of ordinary skill in the art using generally known synthesis techniques. (See for example, Marsh et al., 1949, J. Industrial and Engineering Chemistry, vol. 41, pp. 2176 - 2184; Beaver et al., 1952, J. Amer. Chem. Soc., vol. 74, pp. 3410 - 3411). Exemplary syntheses of bis - phenol compounds are presented below and are not meant to be limiting.

Exemplary Synthesis of Bis - Compounds

Exemplary Synthesis #1

To a solution of 2,4 - dimethyl phenol (24.6 g; 0.2 mol) in CH_3CO_2H (40 ml) and H_2SO_4 (6 ml) cooled with an ice - water bath was added 3,5, - trimethylhexanal (17.1 g; 0.12 mol) in CH_3CO_2H (10 ml) under stirring. The mixture was stirred at room temperature for 10 hrs. The reaction mixture was poured into ice water. The white solid which formed was filtered and washed repeatedly with aqueous ethanol. Recrystallization from aqueous ethanol provided 17.0 g of bis - 1,1 - [2 - hydroxy - 3,5 - dimethylphenyl] - 3,5,5 - dimethyl hexan, which has a melting point (m.p.) of 168 ℃).

Exemplary Synthesis #2

To a solution of 2 - chloro 4 - methyl phenol (28.5 g; 0.2 mol) in CH_3C0_2H (40 ml) and H_2S0_4 (6 ml) was added nonyl aldehyde (16g) in CH_3C0_2H (5 ml) under stirring. The mixture was stirred at 80 ℃ overnight. The reaction mixture was poured into ice water and extracted three times with ethyl acetate. The combined organic phases were washed with 10% $NaC0_3$ and brine, dried, filtered through a pad of silica gel 60. Chromatography (10% ethyl acetate in hexane) provided 12.4 g of pure bis - 1,1 - [2 - hydroxy - 3 - chloro - 5 - methylphenyl]nonane, which has a m.p. of 127 ℃ .

A significant number of bis - compounds have been synthesized, and the chemical formula of these compounds is presented in tabular form in Table 1 below. R1 - R12 correspond to those symbols in the structural formulae set forth above.

Table 1. Bis - compounds

	R1	R2	R3	R4	R5	R6
53433	H	OH	t - $(CH3)_3C$	H	CH3	CH3
53437	H	OH	t - C8H17	H	CH3	CH3
54372	H	OC4H7	t - C8H17	H	CH3	CH3
51850	OH	CH3	H	CH3	H	H
51851	OH	CH3	H	CH3	H	n - C3H7
54013	OH	CH3	H	CH3	H	isoC3H7
51852	OH	CH3	H	CH3	H	C7H16
51853	OH	CH3	H	CH3	H	C8H17
54015	OH	CH3	H	CH3	H	C6H13
54019	OH	CH3	H	CH3	H	C5H11
8302	OH	CH3	H	CH3	H	C9H19
54005	OH	t - C4H9	H	CH3	H	CH3
54008	OH	t - C4H9	H	CH3	H	C2H5
54016	OH	t - C4H9	H	CH3	H	C2H5 - Ph
54011	OH	t - C4H9	H	CH3	H	Ph
54018	OH	t - C4H9	H	CH3	H	C5H11

54020	OH	t - C4H9	H	CH3	H	C4H9
54014	OH	t - C4H9	H	CH3	H	nC6H13
54011	OH	t - C4H9	H	CH3	H	nC7H15
54030	OH	t - C4H9	H	t - C4H9	H	H
54332	OH	t - C4H9	H	t - C4H9	H	C2H5
54107	OH	t - C4H9	H	t - C4H9	H	CH3
54002	OH	t - C4H9	H	t - C4H9	H	C3H7
54040	OH	t - C4H9	H	t - C4H9	H	C4H9
54047	OH	t - C4H9	H	t - C4H9	H	Ph
54041	OH	t - C4H9	H	t - C4H9	H	n - C7H15
54333	OH	C(CH3)2 - CH2CH3	H	C(CH3)2 - CH2CH3	H	C3H7
8296	OH	CH3	H	Cl	H	H
60109	H	t - C4H9	OH	t - C4H9	H	H
54085	H	t - C4H9	OH	t - C4H9	H	CH3
54029	H	t - C4H9	OH	t - C4H9	H	C2H5
54032	H	t - C4H9	OH	t - C4H9	H	nC3H7
54051	H	t - C4H9	OH	t - C4H9	H	Ph
54055	H	t - C4H9	OH	t - C4H9	H	Ph - pCO2H
60634	H	t - C4H9	OH	t - C4H9	CH3	CH3
54483	H	t - C4H9	OH	H	H	isoC3H7
54093	H	CH3	OH	H	CH3	CH3
54094	H	CH3	OH	H	C6H13	CH3
54455	H	CH3	OH	H	C2H5	C2H5
54096	H	CH3	OH	H	- (CH2)4 -	- (CH2)4 -
54099	H	CH3	OH	H	nC3H7	nC3H7
54453	H	CH3	OH	H	CH3	isoButyl
54454	H	CH3	OH	H	CH3	isoC3H7
54063	H	CH3	OH	H	CH3	nC3H7

54059	H	CH3	OH	H	CH3	nC6H13
54009	H	CH3	OH	H	CH3	C3H7
54003	H	CH3	OH	H	CH3	C2H5
54061	H	CH3	OH	H	nC3H7	nC3H7
54031	H	CH3	OH	H	CH3	C2H5
54045	H	CH3	OH	H	C2H5	C2H5
54048	H	CH3	OH	H	-(CH2)5-	-(CH2)5-
54052	H	CH3	OH	H	CH3	isoButyl
54037	H	CH3	OH	CH3	H	H
54046	H	CH3	OH	CH3	CH3	CH3
54471	H	CH3	OH	CH3	H	C2H5
54034	H	CH3	OH	CH3	H	CH3
54028	H	CH3	OH	CH3	C2H5	C2H5
52782	H	CH3	OH	CH3	CH3	C4H9
54044	H	CH3	OH	CH3	H	nC3H7
54098	H	CH3	OH	CH3	H	isoC3H7
54056	H	CH3	OH	CH3	CH3	C2H5
54060	H	CH3	OH	CH3	H	Ph
54097	H	CH3	OH	CH3	H	Ph - pCH3
54095	H	CH3	OH	CH3	H	Ph - pBr
54114	H	CH3	OH	CH3	H	Ph - isoC3H7
54105	H	CH3	OH	CH3	H	CCl3
54042	H	CH3	OH	CH3	H	Ph - pCl
54470	H	CH3	OH	CH3	H	Ph - pOH
54001	H	CH3	OH	CH3	H	Ph - mNO2
54101	H	CH3	OH	CH3	H	Ph - pNO2
54038	H	CH3	OH	CH3	H	Ph - mOH (- pOCH3)

54004	H	CH3	OH	CH3	H	Ph - mOH (- pOH)
54006	H	CH3	OH	CH3	H	Ph - mOCH3 (- pOH)
54043	H	CH3	OH	CH3	H	Ph - (- O(CH2) - O)
54102	H	CH3	OH	CH3	H	Ph - mOH
54064	H	CH3	OH	CH3	CH3	C3H7
54474	H	CH3	OH	CH3	CH3	isoButyl
54028	H	CH3	OH	CH3	C2H5	C2H5
54035	H	CH3	OH	CH3	p - tolyl	p - tolyl
54046	H	CH3	OH	CH3	CH3	CH3
54049	H	CH3	OH	CH3	H	Ph - mCl
54050	H	CH3	OH	CH3	H	Ph - p - CO2H
54057	H	CH3	OH	CH3	H	Ph - p - CO2CH3
54023	H	CH3	OH	CH3	- (CH2)5 -	- (CH2)5 -
54109	H	iso - C3H7	OH	CH3	H	C2H5
54054	H	t - C4H9	OH	CH3	CH3	H
54472	H	t - C4H9	OH	CH3	H	C2H5
54346	H	t - C4H9	OH	CH3	H	Ph - CO2H
54022	H	iso - C3H7	OH	CH3	isoC3H7	H
54021	H	t - C4H9	OH	CH3	CH3	CH3
54053	H	t - C4H9	OH	CH3	H	Ph - p - NO2
54058	H	t - C4H9	OH	CH3	H	Ph
54012	H	isoC3H7	OH	H	H	C3H7
54017	H	isoC3H7	OH	H	H	isoC3H7
54109	H	isoC3H7	OH	H	H	C2H5
54007	H	isoC3H7	OH	H	H	Ph
54024	H	isoC3H7	OH	H	H	CH3

8317	H	CH3	OH	CH3	H	2 - ethyl 4,4 dimethyl - pentyl
8335	OH	CH3	H	CH3	H	C8H17
8334	H	CH3	OH	CH3	H	C8H17
8316	OH	H	Cl	CH3	H	2 - methyl - 4,4 - dimethyl - pentyl
51853	OH	CH3	H	CH3	H	2 - methyl 4,4 - dimethyl pentyl
1	OH	CH3	H	CH3	H	2,6 dimethyl - hept - 5 - ene
2	OH	CH3	H	CH3	H	2,6 dimethyl 6 methoxy - heptyl
3	OH	CH3	H	CH3	H	2,6 dimethy - heptyl
4	OH	CH3	H	CH3	H	2,6 dimethyl - heptane
5	OH	CH3	H	CH3	H	6 methoxy 2,6 - dimethyl - heptane
6	OH	CH3	H	CH3	H	2,6 dimethyl - hept - 5 - ene
7	H	CH3	OH	CH3	H	2,6 dimethyl heptane
8	H	CH3	OH	CH3	H	6 methoxy 2,6 dimethyl heptane
9	H	CH3	OH	CH3	H	2,6 dimethyl - 5 - heptene

	R7	R8	R9	R10	R11	R12
53433	H	OH	t-$(CH_3)_3C$	H	-O-	
53437	H	OH	t-C8H17	H	-O-	
54372	H	O-n-C4H7	t-C8H17	H	-O-	
51850	OH	CH3	H	CH3	H	H
51851	OH	CH3	H	CH3	H	H
54013	OH	CH3	H	CH3	H	H
51852	OH	CH3	H	CH3	H	H
51853	OH	CH3	H	CH3	H	H
54015	OH	CH3	H	CH3	H	H
54019	OH	CH3	H	CH3	H	H
8302	OH	CH3	H	CH3	H	H
54005	OH	t-C4H9	H	CH3	H	H
54008	OH	t-C4H9	H	CH3	H	H
54016	OH	t-C4H9	H	CH3	H	H
54011	OH	t-C4H9	H	CH3	H	H
54018	OH	t-C4H9	H	CH3	H	H
54020	OH	t-C4H9	H	CH3	H	H
54014	OH	t-C4H9	H	CH3	H	H
54011	OH	t-C4H9	H	CH3	H	H
54030	OH	t-C4H9	H	t-C4H9	H	H
54332	OH	t-C4H9	H	t-C4H9	H	H
54107	OH	t-C4H9	H	t-C4H9	H	H
54002	OH	t-C4H9	H	t-C4H9	H	H
54040	OH	t-C4H9	H	t-C4H9	H	H
54047	OH	t-C4H9	H	t-C4H9	H	H
54041	OH	t-C4H9	H	t-C4H9	H	H
54333	OH	$C(CH_3)_2$-CH2CH3	H	$C(CH_3)_2$-CH2CH3	H	H

8296	OH	CH3	H	Cl	H	H
60109	H	t - C4H9	OH	t - C4H9	H	H
54085	H	t - C4H9	OH	t - C4H9	H	H
54029	H	t - C4H9	OH	t - C4H9	H	H
54032	H	t - C4H9	OH	t - C4H9	H	H
54051	H	t - C4H9	OH	t - C4H9	H	H
54055	H	t - C4H9	OH	t - C4H9	H	H
60634	H	t - C4H9	OH	t - C4H9	H	H
54483	H	t - C4H9	OH	t - C4H9	H	H
54093	H	CH3	OH	H	H	H
54094	H	CH3	OH	H	H	H
54455	H	CH3	OH	H	H	H
54096	H	CH3	OH	H	H	H
54099	H	H	OH	H	H	H
54453	H	H	OH	H	H	H
54454	H	H	OH	H	H	H
54063	H	H	OH	H	H	H
54059	H	H	OH	H	H	H
54009	H	CH3	OH	H	H	H
54003	H	CH3	OH	H	H	H
54061	H	CH3	OH	H	H	H
54031	H	H	OH	H	H	H
54045	H	H	OH	H	H	H
54048	H	CH3	OH	H	H	H
54052	H	CH3	OH	H	H	H
54037	H	CH3	OH	CH3	H	H
54046	H	CH3	OH	CH3	H	H
54471	H	CH3	OH	CH3	H	H
54034	H	CH3	OH	CH3	H	H

54028	H	CH3	OH	CH3	H	H
52782	H	CH3	OH	CH3	H	H
54044	H	CH3	OH	CH3	H	H
54098	H	CH3	OH	CH3	H	H
54056	H	CH3	OH	CH3	H	H
54060	H	CH3	OH	CH3	H	H
54097	H	CH3	OH	CH3	H	H
54095	H	CH3	OH	CH3	H	H
54114	H	CH3	OH	CH3	H	H
54105	H	CH3	OH	CH3	H	H
54042	H	CH3	OH	CH3	H	H
54470	H	CH3	OH	CH3	H	H
54001	H	CH3	OH	CH3	H	H
54101	H	CH3	OH	CH3	H	H
54038	H	CH3	OH	CH3	H	H
54004	H	CH3	OH	CH3	H	H
54006	H	CH3	OH	CH3	H	H
54043	H	CH3	OH	CH3	H	H
54102	H	CH3	OH	CH3	H	H
54064	H	CH3	OH	CH3	H	H
54474	H	CH3	OH	CH3	H	H
54028	H	CH3	OH	CH3	H	H
54035	H	CH3	OH	CH3	H	H
54046	H	CH3	OH	CH3	H	H
54049	H	CH3	OH	CH3	H	H
54050	H	CH3	OH	CH3	H	H
54057	H	CH3	OH	CH3	H	H
54023	H	CH3	OH	CH3	H	H
54109	H	isoC3H7	OH	CH3	H	H

54054	H	t - C4H9	OH	CH3	H	H
54472	H	t - C4H9	OH	CH3	H	H
54346	H	t - C4H9	OH	CH3	H	H
54022	H	iso - C3H7	OH	CH3	H	H
54021	H	t - C4H9	OH	CH3	H	H
54053	H	t - C4H9	OH	CH3	H	H
54058	H	t - C4H9	OH	CH3	H	H
54012	H	isoC3H7	OH	H	CH3	CH3
54017	H	isoC3H7	OH	H	CH3	CH3
54109	H	isoC3H7	OH	H	CH3	CH3
54007	H	isoC3H7	OH	H	CH3	CH3
54024	H	isoC3H7	OH	H	CH3	CH3
8317	H	CH3	OH	CH3	H	H
8335	OH	CH3	H	CH3	H	H
8334	H	CH3	OH	CH3	H	H
8316	OH	H	Cl	CH3	H	H
51853	OH	CH3	H	CH3	H	H
1	OH	CH3	H	CH3	H	H
2	OH	CH3	H	CH3	H	H
3	OH	CH3	H	CH3	H	H
4	OH	CH3	H	CH3	H	H
5	OH	CH3	H	CH3	H	H
6	OH	CH3	H	CH3	H	H
7	H	CH3	OH	CH3	H	H
8	H	CH3	OH	CH3	H	H
9	H	CH3	OH	CH3	H	H

Compounds which antagonize IL - 12 can be identified readily using the assays described below. The described *in vitro* Screening Assay provides for the rapid screening of large numbers of compounds for their ability to antagonize IL - 12, as measured by the

inhibition of IFN - γ production. The screening assay described below also may be automated. Compounds which are identified by the screening assay may then be screened by a Mixed Lymphocyte Reaction Assay to analyze the compound further for its activity as either a general immune cell suppressor or as an inhibitor of IL - 12 induced production of a Th1 immune response. Promising compounds, those which are not general immune suppressors, are tested for *in vivo* toxicity in rats.

The invention is illustrated further by the following non - limiting examples:

Example 1 Screening Assay for Compounds which Inhibit IL - 12 Induction of IFN - γ .

Human peripheral blood monocytes (PBMC) were obtained from commercial sources as a leukophoresis from a healthy volunteer and were purified by centrifugation on a Ficoll - Hypaque (Pharmacia) gradient (1700 rpm for 20 min). The "buffy" coat containing the PBMC was diluted with serum - free culture medium (SF - RPMI) to a volume of 50 ml and collected by centrifugation at 1500 rpm for 5 min. Cells were resuspended in cell culture medium containing 10% fetal bovine serum (RPMI - 10) and phytohemaglutinin (PHA - 10 μ g/ml) at a density of 5 x 10^6 cells/ml and were cultured for 3 days at 37℃ in a humidified CO_2 incubator. The PHA - activated cells were collected by centrifugation, washed three times with an equal volume of SF - RPMI and resuspended in fresh RPMI - 10 (1 x 10^6 cells /ml). Aliquots (100 μ l) were dispensed into the wells of multiple 96 - well plates to give a final cell number of 10^5 per well. Test compounds, dissolved in dimethyl sulfoxide (DMSO) at 1 mg/ml, were first diluted in culture medium to an intermediate concentration of 20 μ g/ml and then were added (50 μ l/well) to a specific well of the plate for each compound. Stimulation medium (50 μ l/well) containing 10% serum, IL - 2 and IL - 12 was added to final concentrations of 25 U/ml and 0.5 ng/ml, respectively. Control wells receive no IL - 2 or IL - 12 (negative control) or received both interleukins but no test compound (positive control). The plates were incubated for 48 hr at 37 ℃ in a CO_2 incubator at which time aliquots (20 μ l) were removed for analysis of IFN - γ concentration by ELISA. A quantitative ELISA was developed by coating 96 - well plates with an mouse monoclonal antibody against human

IFN - γ , 1 μ g/ml in phosphate buffered saline (PBS) (Pestka Biological Laboratories), overnight at 4 ℃ . Unbound antibody was washed off by washing three times with PBS. Non - specific antibody binding was blocked with a solution of 1% bovine serum albumin (BSA) and 1% goat serum in PBS (150 μ l/well) which was incubated for 2 hr at 37 ℃ . After washing the blocked plates four times with PBS, test samples and dilutions of the IFN - γ standard are added in a final volume of 100 μ l/well. Following an overnight incubation at 4 ℃ , the plates are washed four times with PBS, and a polyclonal rabbit antiserum against human IFN - γ (1/10000 dilution - Petska Biological Laboratories) is added. After an additional incubation for 1 hr at 37 ℃ and four washes with PBS, a polyclonal donkey anti - rabbit detecting antibody, conjugated to horseradish peroxidase (1/700 dilution - Petska Biological Laboratories) is added for 1 hr at 37 ℃ . The plates are then washed four times with PBS and 100 μ l of K - blue substrate (ELISA Technologies, Neogen Corp.) is added until the color in the wells containing the standard curve is sufficiently developed, at which time 100 μ l of "Red - stop" solution (ELISA Technologies) is added. The absorbance of the solution within each well of the plate is then read at 650 nm using an ELISA plate reader (Dynatech MR7000). The amount of IFN - γ is calculated by comparing the optical density of the test sample with a standard curve derived from the dilutions of the control IFN - γ . The amount of IFN - γ that is induced in the presence of both IL - 2 and IL - 12 generally ranges from 1200 - 2000 pg/ml while the amount produced in the absence of IL - 12 is generally less than 50 pg/ml. Experimental data are shown in Table 2 which discloses a) the percent inhibition of IFN γ production, relative to a negative control of untreated cells, when the cells have been treated with a bis - compound at a final concentration of 5 μ g/ml of the listed bis - compound (%INH of IFN γ) and b) the percent of cells which are viable after the treatment with 5 μ g/ml of the various bis - compounds, determined by adding MTS to the media (% Viability). MTS is a chemical chromophore that is metabolized in the mitochondria of viable cells to produce a color which increases in intensity in proportion to the numbers of viable cells. The absorbance of the cell culture can be compared to control cultures in order to determine the percent viability.

Table 2.

Compound ID #	% INH of IFN γ	% Viability
8296	103	37
8302	95	100
51850	77	97
51851	86	107
51852	109	105
51853	104	104
52782	102	116
53433	97	26
53437	74	85
53529	18	88
53553	62	72
54003	101	85
54004	- 37	99
54005	96	30
54007	95	26
54008	91	69
54010	69	97
54015	96	34
54016	26	98
54018	55	92
54019	77	101
54020	69	95
54025	78	48
54028	96	101
54036	34	92
54039	78	78

54042	28	102
54044	98	92
54046	76	95
54048	68	97
54050	63	86
54053	93	33
54054	98	27
54055	83	88
54056	84	87
54058	88	86
54059	64	88
54062	92	58
54063	73	93
54064	79	112
54094	97	25
54099	85	93
54109	93	33
54113	96	74
54114	65	96
54346	91	84
54471	89	83
54472	98	26

As can be seen from the data, several of the bis- compounds were effective at inhibiting IFN- γ production while not decreasing the viability of the cells. The data presented were obtained from a single test of each listed compound in the above described assay. Generally, each compound which appears to be of interest, is tested two or more times using the above assay and an average of the data results is calculated. A compound which inhibits IFN- γ production by approximately 70% while maintaining cell viability at approximately 70% or higher is considered to be of interest for further screening.

Example 2 Mixed Lymphocyte Reaction

This reaction measures the T cell response to allogenic stimulation by a mismatch of class II histocompatability antigens. This is triggered through the T cell receptor and activates the T cell to secrete and proliferate in response to endogenous IL-2. IFN-γ is also made in response to this stimulation and accumulates in the culture medium. Compounds are tested for their ability to block the proliferation and response to antigenic stimulation as well as their ability to produce IFN-γ as follows. A human B cell line expressing class II antigen, Namalwa (American Type Culture Collection), is incubated for 2 hr at 37 ℃ in RPMI-10 containing mitomycin C (50 μ g/ml). These cells are washed four times by centrifugation and resuspension (10^6 cells/ml) in SF-RPMI medium and are added to the wells of 96-well plate (50 μ l/well) as stimulators. Mitomycin treatment prevents these cells from proliferating in the assay. Fresh PBMC are prepared as described above in example 1, but are not activated with PHA. Instead they are resuspended at 10^6 cells/ml and 100 μ l is added per well. Dilutions of test compounds are added (50 μ l/well) to give a final volume of 200 μ l and the plates are incubated for 6 days at 37℃. During the last 18 hr, ^{3}H-thymidine (DuPont-NEN-1 μ Ci/well) is added as a measure of cell proliferation, cultures are collected using a cell harvester (Packard), and scintillation fluid is added prior to counting in a Packard Top Count scintillation counter. A sample of medium is also collected for measurement of IFN-γ production prior to the addition of ^{3}H-thymidine for use as a negative control.

Representative data are shown in Figures 1-3 for two bis-phenol compounds, compounds 51853 and 51852, and for 8-azaguanine, which is another class of inhibitor of IL-12 induced IFN-γ secretion that is a general immunosuppressant. A general immunosuppressant is a compound which inhibits both proliferation and IFN-γ secretion equally. The bis-compounds, on the other hand, are more potent inhibitors of secretion at concentrations that do not effect cell proliferation, and thus are shown to be specific inhibitors of Th1 proliferation.

Example 3 Toxicity of Bis-Compounds

The potential for toxic side effects was assessed following systemic

administration of a single dose of bis - compound to mice by an intraperitoneal injection . The results are summarized in Table 3 below.

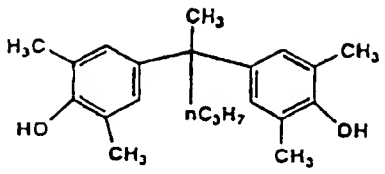
Toxicity Report

Comp.	Solution	Inj	Dose mg/kg	Conc. sol. mg/ml	Results	LD50
51853	20% DMSO 16% cremaphor	IV	150 100	10	Dead Alive	>100<150
52782	20% DMSO 16% cremaphor	IV	80	10	Dead	<80
54015	20% DMSO 16% cremaphor	IV	80	10	Alive	>80
54044	20% DMSO 16% cremaphor	IV	150 100	10	Alive Alive	>150
54064	20% DMSO 16% cremaphor	IV	150 100	10	Dead Alive	>100<150
54039	20% DMSO 16% cremaphor	IV	150 100	10	Dead Dead	<100
54016	20% DMSO 16% cremaphor	IV	150 100	10	Alive Alive	>150
54028	20% DMSO 16% cremaphor	IV	150 100	10	Alive Alive	>150
51850	20% DMSO 16% cremaphor	IV	150 100	10	Alive Alive	>150
54019	20% DMSO 16% cremaphor	IV	150 100	10	Dead Alive	>100<150
54003	20% DMSO 16% cremaphor	IV	150 100	10	Dead Alive	>100<150
54046	20% DMSO 16% cremaphor	IV	150 100	10	Alive Alive	>150
54018	20% EGME 16% cremaphor	IV	150 100	10	Dead Dead	<100
54020	20% EGME 16% cremaphor	IV	150 100	10	Dead Alive	>100<150

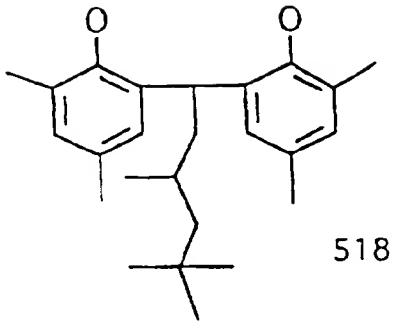
Comp.	Solution	Inj	Dose mg/kg	Conc. sol. mg/ml	Results	LD50
8302	20% DMSO 16% cremaphor	IV	100 150	10 10	Alive Alive	>150
51853	20% DMSO 16% cremaphor	IV	200 150	15	Alive Alive	>200
8302	16% cremaphore 20% DMSO	IV	200	15	Alive 1 Dead 1	200

Based upon the combined results of the above described examples, a subset of the bis - compounds were identified to be potentially useful as compounds for the manufacture of pharmaceuticals for administration to mammals for the purpose of antagonizing IL - 12. It is envisioned that such bis - compounds may be administered to humans orally or parenterally for the treatment of multiple sclerosis. Further that bis - compounds may be used topically for the treatment of skin diseases, such as psoriasis. It is envisioned also that bis - compounds can be used in combination with other immunosuppressive drugs such as Cyclosporin A, for example, in organ transplantation to improve the effectiveness of immune modulation and reduce the dose of Cyclosporin A required, thereby decreasing potential toxicity to the subject.

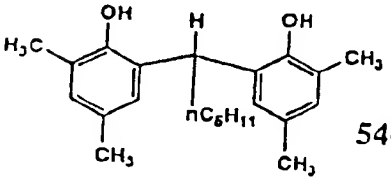
The currently preferred bis - compounds for formulation as compositions to antagonize IL - 12 include the compounds designated as 8302, 51853, 51850, 54020, 54019, 54003, 54028, 54064, 54044, and 54046. These compounds have the chemical structures presented below.



54064



51853



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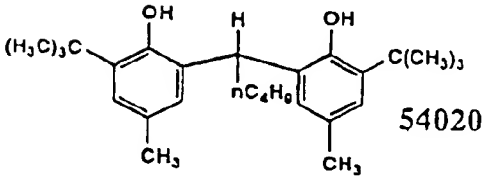
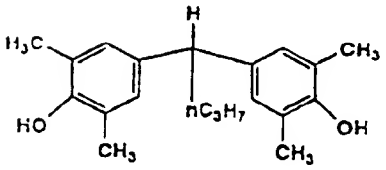
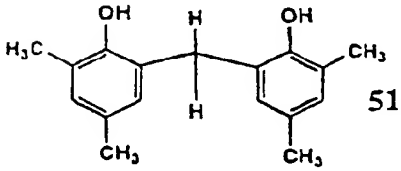


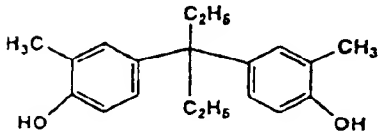
54020



54044



51850



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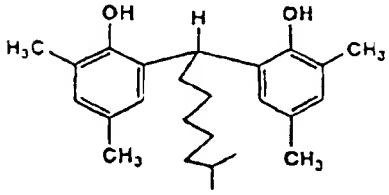
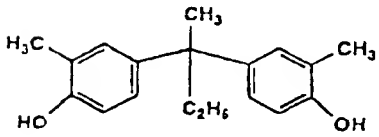
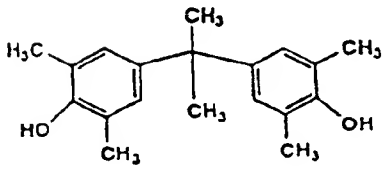


8302



54003



54046

Example 4. *In Vitro* Diagnostic for IL4 Production

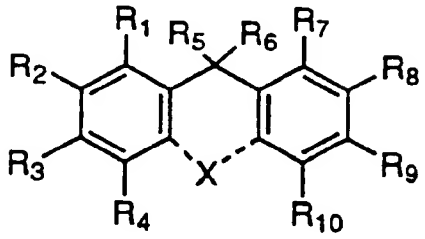
The ability of immune cells to produce IL4 *in vitro* can be measured according to the following assay. A measurement of the production of IL4 by immune cells *in vitro* will allow a determination of a subject's ability to produce a Th2 immune response. This serves as a measure of predisposition to allergy.

Human peripheral blood monocytes (PBMCs) are obtained from the subject to be tested and purified as described above. The PBMCs are divided into aliquots and are stimulated to proliferate with phytohemaglutinin either in the presence or absence of a bis- compound. Addition of bis - compound to an aliquot of stimulated PBMCs will inhibit INF γ production and provide for a true response of IL4 production to be measured. For instance, if a test subject has a concurrent viral infection, such that INF γ production was stimulated prior to obtaining the PBMC sample, then the addition of bis - compound to the PBMC aliquot would inhibit further IL12 induced INF γ production and allow for an accurate measure of IL4 production. The generation of a standard IL4 production response curve from a number of individuals will allow the determination of whether the IL4 production of the test subject falls within normal ranges. If the test subject has a lower than normal IL4 production response in stimulated PBMCs then it may be that the Th2 immune response in the individual is prevented by an immune cell disorder. If the IL4 production response is higher than normal, then it may be that the subject individual is predisposed to allergic reaction.

Other embodiments of the invention will be apparent to those skilled in the art from a consideration of this specification or practice of the invention herein disclosed. It is intended that the specification be construed as exemplary only, with the true scope and spirit of the invention represented by the following claims.

CLAIMS

1. A composition for antagonizing the IL - 12 induced immune response comprising a pharmaceutically acceptable carrier and a bis - compound having the formula:



wherein X is optional, and if present, is - O - , - S - , or - CH_2 - ;

at least one of R_1, R_2, R_3, and R_4 and at least one of R_7, R_8, R_9, and R_{10} is OR_{13}, where R_{13} is H or lower alkyl;

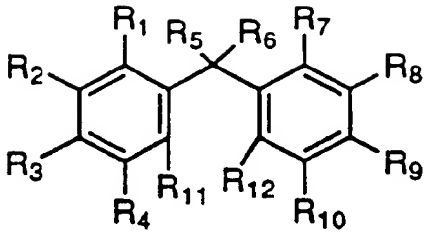
R_5 and R_6 are selected independently from H, C_1 - C_{12} branched or linear hydrocarbons, phenyl, phenyl substituted with halo, nitro, carboxy, alkoxy, hydroxy, alkoxy carbonyl, or alkyl, alkyl substituted with halo, nitro, carboxy, alkoxy, hydroxy, or alkoxy carbonyl, or alkenyl, alkenyl substituted with halo, nitro, carboxy, alkoxy, hydroxy, or alkoxy carbonyl;

and

each of R_1, R_2, R_3, R_4, R_7, R_8, R_9 and R_{10} which is not OR_{13} is independently hydrogen, halo, or linear or branched lower alkyl.

2. The composition of claim 1 wherein X is absent and one of R_1, R_2, R_3, and R_4, and one of R_7, R_8, R_9, and R_{10} is hydroxyl.

3. A composition for antagonizing the IL - 12 induced immune response comprising a pharmaceutically acceptable carrier and a bis - compound having the formula:



wherein at least one of R_1, R_2, R_3, R_4, and R_{11} and at least one of R_7, R_8, R_9, R_{10}, and R_{12}, is OR_{13}, where R_{13} is H or lower alkyl;

R_5 and R_6 are selected independently from H, C_1 - C_{12} branched or linear hydrocarbons, phenyl, phenyl substituted with halo, nitro, carboxy, alkoxy, hydroxy, alkoxy carbonyl, or alkyl, alkyl substituted with halo, nitro, carboxy, alkoxy, hydroxy, or alkoxy carbonyl, or alkenyl, alkenyl substituted with halo, nitro, carboxy, alkoxy, hydroxy, or alkoxy carbonyl;

and

each of R_1, R_2, R_3, R_4, R_7, R_8, R_9, R_{10}, R_{11} and R_{12} which is not OH or OR_{13} is independently hydrogen, halo, or linear or branched lower alkyl.

4. The composition of claim 3 wherein R_5, R_{11}, R_{12} are H, R_6 is a C_1 - C_{12} branched or linear hydrocarbon, and one of R_1, R_2, R_3, and R_4, and one of R_7, R_8, R_9, and R_{10}, is hydroxyl.

5. The composition of claim 3 wherein at least one of R_1, R_2, R_3, R_4, and R_{11} and at least one of R_7, R_8, R_9, R_{10}, and R_{12}, is OH; and

one or both of R_5 and R_6 is H or a hydrocarbon having 1 to 12 carbon atoms.

6. The composition of claim 5 wherein one of R_5 and R_6 is H and the other is a branched alkyl chain having 1 to 12 carbon atoms.

7. The composition of claim 5 wherein each R_1, R_2, R_3, R_4, R_7, R_8, R_9, R_{10}, R_{11} and R_{12} which is not OH is hydrogen, methyl, ethyl, propyl, isopropyl, butyl, tertiary - butyl, or

linear or branched pentyl.

8. The composition of any one of claims 1 to 7 wherein the IL - 12 induced immune response is the IL - 12 mediated induction of IFN - γ synthesis.

9. The composition of any one of claims 1 to 7 which inhibits the induction of Th1 cells.

10. The composition of any one of claims 1 to 7 which modulates the induction of Th2 cells.

11. The composition of any one of claims 1 to 7 which inhibits the cellular immune response.

12. The composition of any one of claims 1 to 7 which stimulates the production of Th2 cylokines including IL - 4, IL - 5, or IL - 10.

13. The composition of any one of claims 1 to 12 used for preventine and/or therapeutic treatment of an autoimmune disease.

14. The composition of any one of claims 1 to 13 comprising a pharmaceutically acceptable carrier and a bis - compound having the formula of either 8302, 51853, 51850, 54020, 54019, 54003, 54028, 54064, 54044 or 54046.

15. A use of the compound of any one of claims 1 to 7 for a manufacture of the composition according to any one of claims 1 to 13.

16. A method of for preventine and/or therapeutic treatment of an autoimmune disease comprising the step of administering to a mammal the compound of any one of

claims 1 to 7 in an amount effective to antagonize IL - 12 mediated induction of IFN - γ synthesis.

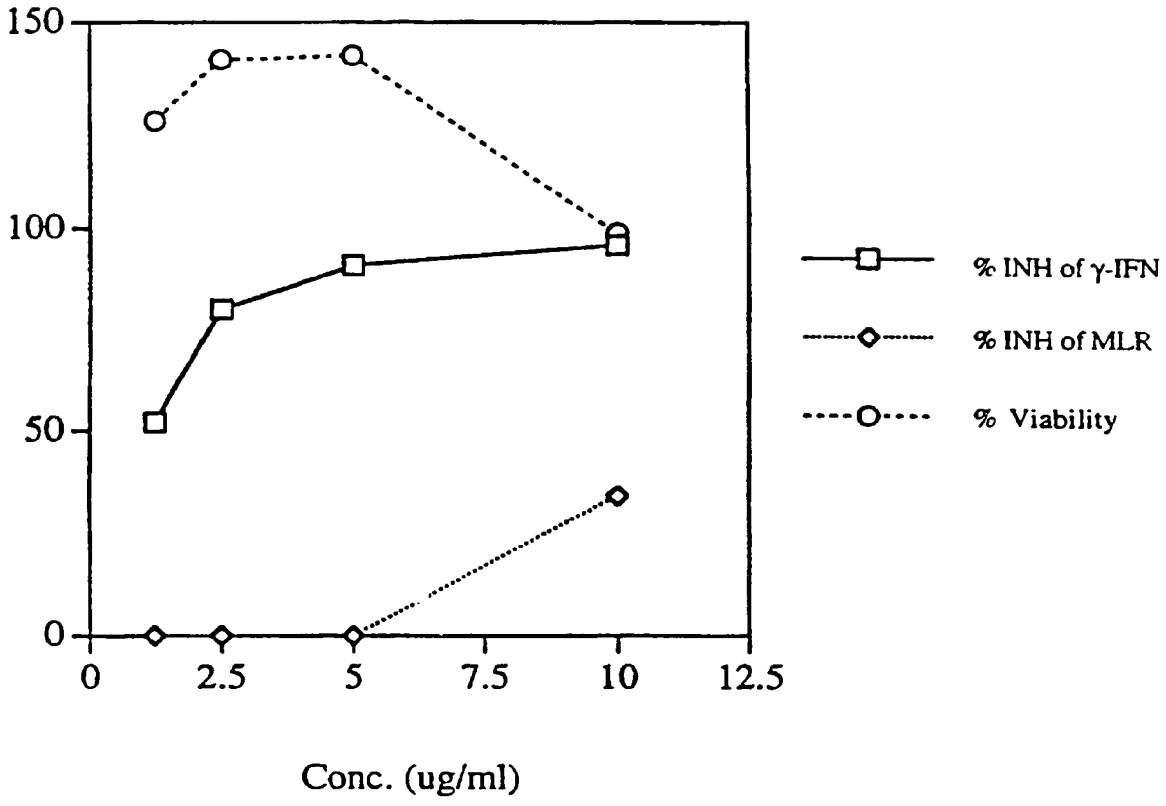


Fig.1

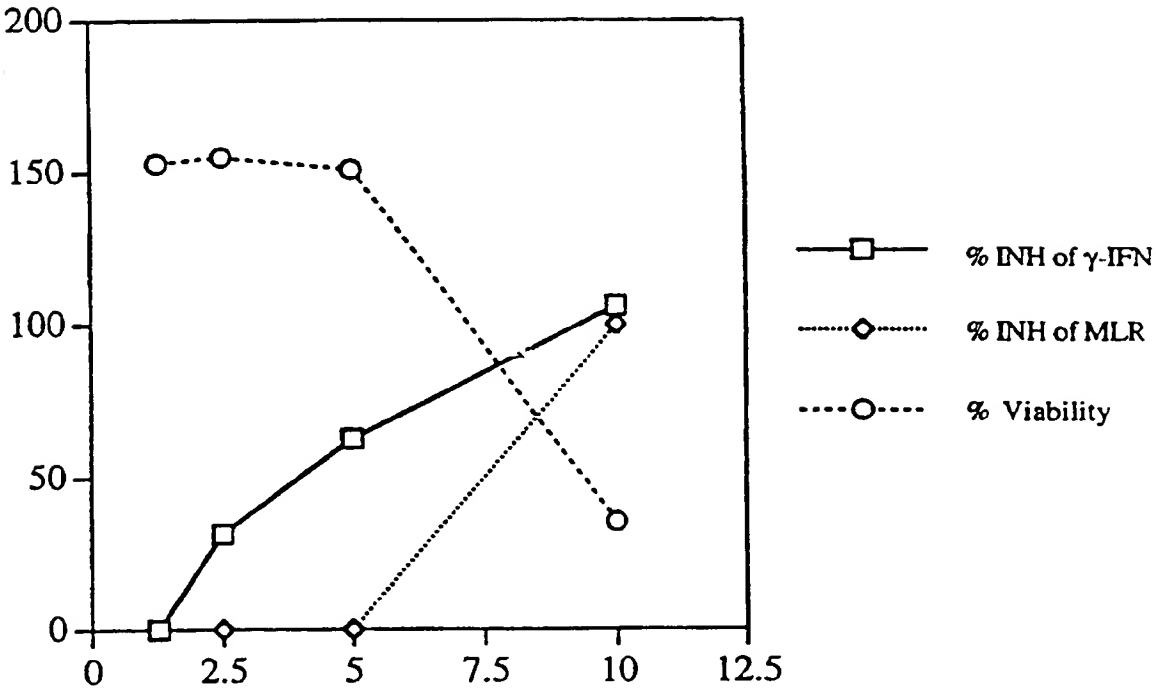


Fig.2

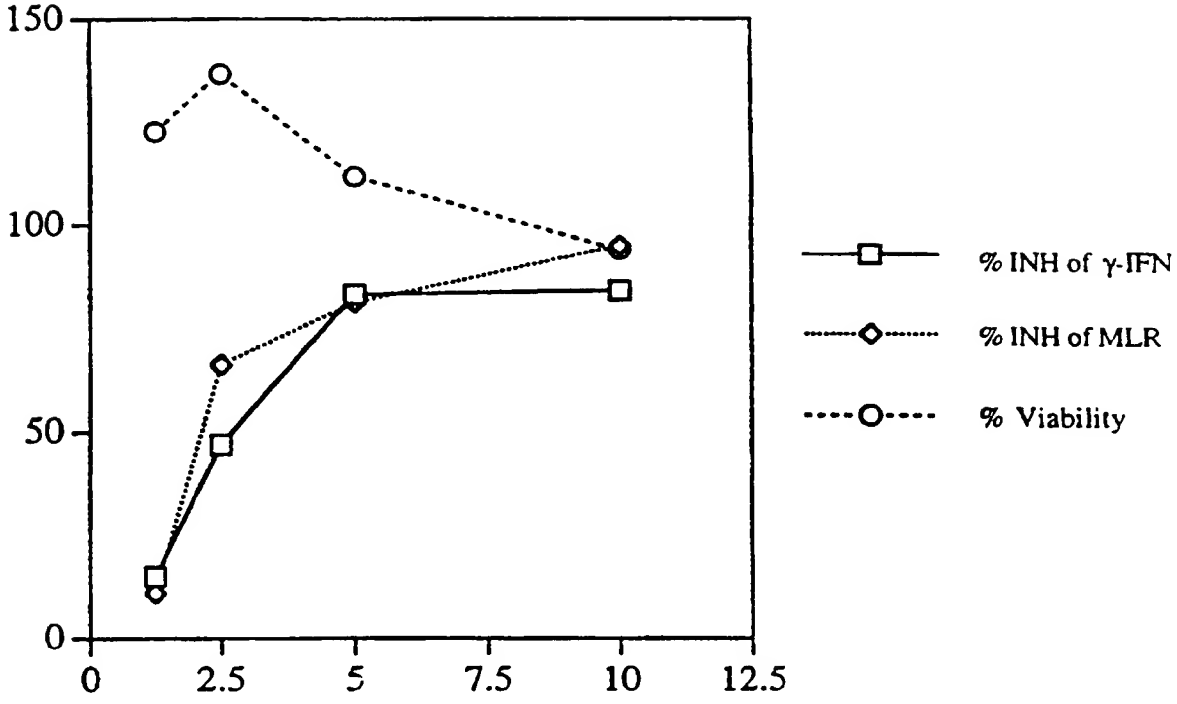


Fig.3

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(54) **Title:** BIS-PHENOL OR PHENOXY COMPOUNDS FOR IMMUNE MODULATION

(57) **Abstract**

Disclosed are chemical agents for modulating certain cellular immune reactions that can lead to autoimmune disorders. By specific modulation, harmful immune reactions can be lessened in severity or even prevented without resorting to potentially dangerous general immune suppression. The described chemical agents inhibit IL-12 induction of the secretion of key immune modulators. The described chemical agents are specific inhibitors of IL-12 induced Th1 immune response.

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INTERNATIONAL SEARCH REPORT

Inte onal Application No
PCT/JP 96/01552

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K31/05

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 583 665 A (FUJI PHOTO FILM CO LTD) 23 February 1994 see the whole document especially compound 54 ---	1-15
X	JOURNAL OF DENTAL RESEARCH, vol. 74, no. 5, May 1995, pages 1162-1167, XP000617048 JONTELL, M. ET AL: "Effects of unpolymerized resin components on the function of accessory cells derived from the rat incisor pulp"	1-3,5, 7-13,15
A	see the whole document especially page 1166, figure 7 & page 1163, lefthand column, line 37-righthand column, line 11 --- -/--	16

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
31 January 1997	11.02.97

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Mair, J

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INTERNATIONAL SEARCH REPORT

Inte onal Application No
PCT/JP 96/01552

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	YUKAGAKU (JOURNAL JAPANESE OIL CHEM. SOC.), vol. 44, no. 11, 1995, pages 960-965, XP000617064 NISHIYAMA, TOMIHIRO ET AL: "Antioxidant activity of the Fused heterocyclic compounds, 9H-Xanthene-2,7-diols" see the whole document	1-16
A	JOURNAL OF IMMUNOLOGY, vol. 154, 15 March 1995, pages 2959-68, XP000616368 CORREALE, J. ET AL: "Patterns of cytokine secretion by autoreactive proteolipid protein-specific T cell clones during the course of multiple sclerosis" cited in the application see the whole document especially page 2966, righthand column, line 24-44	1-16
A	NEUROLOGY, vol. 43, no. 4, April 1993, pages 655-661, XP000614982 THE IFNB MS STUDY GROUP: "Interferon beta-1b is effective in relapsing-remitting multiple sclerosis" cited in the application see page 660, left-hand column, line 32-46	1-16
A	PROC. NATL. ACAD. SCI. USA, vol. 92, 23 May 1995, pages 4823-4827, XP000616366 GERMANN, T. ET AL: "Administration of interleukin 12 in combination with type II collagen induces severe arthritis in DBA/1 mice" cited in the application see page 4826, Discussion	1-16

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INTERNATIONAL SEARCH REPORT

I national application No.
PCT/JP 96/01552

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claim(s) 16
is(are) directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [X] Claims Nos.: 1-13,15,16
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

In view of the large number of compounds which are defined by the wording of the claims, the search has been performed on the general idea and compounds mentioned in the examples of the description.

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee. this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int. onal Application No
PCT/JP 96/01552

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A-0583665	23-02-94	JP-A-	6048942	22-02-94
		JP-A-	6080563	22-03-94
		US-A-	5387600	07-02-95